8—The proposed one-filtration method, with hard filter paper, gives more reliable results than those commonly obtained by the present official method for crude fiber.

Oklahoma Agricultural and Mechanical College
Stillwater, Oklahoma

THE APPARENT EFFECT OF ACETIC ACID UPON THE CONSTANTS OF BUTTER FAT

By Clarence Bahlman

Received April 23, 1915

During the course of a chemical and bacteriological examination of Cincinnati's ice cream supply carried on by the laboratory of the Cincinnati Department of Health,[1] it became necessary to examine each sample for percentage of milk fat as well as to determine the presence or absence of foreign fats and oils.

The careful attention required for the estimation of fat by the laborious Roese-Gottlieb method would have made it impossible to examine more than a few samples each day. By the use of a simpler method, the laboratory helper was enabled to arrive at the fat content of 20 or 30 samples within one hour's time.

By this method,[2] 9 grams of the melted ice cream are weighed into the ordinary 10 per cent Babcock milk bottle, 20 cc. of glacial acetic acid added, and the mixture thoroughly shaken: 10 cc. of Babcock sulfuric acid are then added and the bottle again shaken. From this point the sample is treated by the ordinary Babcock method for the estimation of fat in milk. The reading of the fat column in the neck of the bottle, multiplied by two, gives the percentage of butter fat.

With this technique, the charring effect of the sulfuric acid upon the sugar is eliminated. The separated fat has a clear, pale yellow color, is free from sediment and foam, and distinctly separated from the lower acid layer.

As a test for foreign fats and oils, a few drops of the clear fat from the Babcock bottles were placed in the Zeiss butyro-refractometer and the refractive index determined at 40° C. With this instrument, pure fat from cows' milk will give, at 40° C., a reading falling between 40.5 and 44.5 scale divisions.

Practically all of the readings upon the samples, however, were well below the minimum figures for pure butter fat. As a general rule, the readings varied from 38.0 to 41.0, with a few running as low as 36.5 scale divisions.

Since it appeared highly improbable that all of our ice cream manufacturers were practicing adulteration, and in view of the fact that the food chemist places such great reliance upon the refractometer, the abnormal results shown by the samples were the subject of further study.

EXPERIMENTAL

One of the most suspicious samples was selected and about 15 grams of fat isolated, both by ether extraction and by the acetic acid Babcock method. The fat obtained by the latter method was taken from the necks of the bottles with a pipette and filtered through paper.

The fat separated by ether extraction gave results for refractive index and other constants which agreed with those for a pure butter fat. The fat obtained by the acetic acid method, however, gave low readings for refraction value and decidedly high Koettstorfer and Reichert-Meissl numbers, although a normal Hanus iodine figure was obtained.

It would appear from the above that when butter fat is separated from dairy products in the presence of acetic acid, the constants are materially altered. The following experiments were undertaken to determine whether such abnormalities were to be attributed to complex changes or merely to mechanical retention of the acid.

EXPERIMENT I—Nine grams of rich cream of known purity were placed into each of 15 Babcock bottles and 20 cc. of glacial acetic acid added. After mixing, 10 cc. of Babcock sulfuric acid were added and the bottles centrifuged as usual. The separated fats were drawn off, combined, and filtered through paper.

Fat was also separated from the same cream by the usual Babcock method of adding 17.5 cc. of sulfuric acid to 18 grams of cream.

After standing in the refrigerator over night, the well-known constants for butter fat were determined, with results shown in Table I.

Table I

Fat obtained by	Babcock method	Acetic acid method	
Refractometer reading, 40° C	41.8	39.4	
Hanus iodine number	28.9	29.1	
Koettstorfer number	227.0	242.0	[226.0]
Reichert-Meissl number	28.3	44.4	[27.2]
Soluble acids	6.0	8.9	[5.6]
Insoluble acids	86.9	85.6	

It was found that 5 grams of the fat obtained by the acetic acid method retained acetic acid equivalent to 1.9 cc. *N* NaOH. Such acidity would have no effect upon iodine absorption nor upon the weight of insoluble acids, and consequently the two samples gave practically identical results for these determinations. The other constants, however, are based upon alkalimetrical principles and acid held by the fat would naturally affect the results. The figures shown in brackets in Table I were obtained by correcting for the amount of acid retained by the fat.

It will be seen that the corrected results vary from those given by the fat separated by the Babcock method only by amounts that can be attributed to experimental error.

It appears, then, that butter fat obtained by the ordinary Babcock method will give normal results for all of the usual determinations, while fat separated in the presence of strong acetic acid gives results apparently abnormal which, however, become normal when we take into account the acidity due to retained acetic acid.

EXPERIMENT II—Hydrochloric acid is also used for the separation of fat from dairy products. Like acetic acid, this also is a volatile acid and might be expected to be retained by the fat, affecting the results as in the case where acetic acid is used.

Eighteen-gram portions of cream were mixed with

---

[1] See *Amer. Jour. of Public Health*, **4** (1914), 1009–1015.

[2] Lichtenburg, This Journal, **5** (1913), 786.

20 cc. of concentrated hydrochloric acid in Babcock cream bottles. The bottles were immersed in boiling water for 5 minutes, after which the fat was separated by the Babcock centrifuge, filtered, and compared with the fat obtained upon the same cream as in the regular Babcock method. The results are shown in Table II.

Table II

Fat obtained by	Babcock method	Hydrochloric acid method
Refractometer reading, 40° C.	41.8	41.8
Koettstorfer number	227.0	228.0
Reichert-Meissl number	28.3	29.0

Hydrochloric acid, therefore, was not retained by the fat to any appreciable extent, 5 grams having an acidity equivalent to only 0.5 cc. of 0.1 *N* NaOH. The Koettstorfer and Reichert-Meissl figures were practically identical with those obtained when using sulfuric acid for the decomposition.

Table III—Results from Treatments of Pure Butter

Sample No.	Determination	Untreated	Treated as in Babcock method	Treated as in HCl method	Treated as in Acetic Acid Method: Not Heated	Heated at 90–95° C.: 1/4 hour	1/2 hour	1 hour	2 hours
1	Refractometer	40.7	40.6	40.6	36.9	38.7	39.7	40.6	40.6
	Koettstorfer	226.0	..	227.0	..	..	..	226.0	..
	Reichert-Meissl	29.7	..	29.4	39.5	..	..	29.3	..
2	Refractometer	42.0	42.0	42.0	38.8	40.2	41.0	42.0	..
	Reichert-Meissl	...	..	...	41.6	..	..	28.2	..
3	Refractometer	42.9	43.0	42.9	39.8	41.6	42.8	42.9	..

EXPERIMENT III—In order to further show that acetic acid is the only acid retained to a sufficient extent to affect the refractive index and other constants of butter fat, three samples of pure butter were melted and the filtered fat treated by shaking in a separatory funnel with such volumes of water and acid as approach the proportions of each present in the analysis as ordinarily carried on. The quantities used were as follows:

a—*Babcock Method:* 15 cc. melted fat, 30 cc. water, 40 cc. concentrated sulfuric acid.

b—*Hydrochloric Acid Method:* 15 cc. melted fat, 90 cc. hot water, 100 cc. concentrated hydrochloric acid.

c—*Acetic Sulfuric Acid Method:* 15 cc. melted fat, 30 cc. water, 100 cc. glacial acetic acid, 50 cc. concentrated sulfuric acid.

After shaking thoroughly for 3 minutes, the fat was allowed to come to the top, filtered, and cooled in the refrigerator. The refractometer readings were then taken upon all, and the Koettstorfer and Reichert-Meissl numbers upon a few, of the samples. The results were compared with the constants obtained upon the untreated fats, as shown in Table III. The refractometer readings were also taken upon the fat treated with acetic acid, after standing for certain time intervals in an air oven at 90–95° C.

This table indicates that abnormal results are obtained only in the case of fats separated in the presence of acetic acid. Such results are due to acetic acid being mechanically held by the fat. There is no acetylization. The fat obtained by the acetic acid method after thorough washing to remove acidity and treating as in the determination of the acetyl value, but without acetylating with acetic anhydride, gives results which do not differ from those upon the untreated fat.

The refraction reading and other constants are the most erratic when the greatest amount of acetic acid is present, but approach nearer to the correct results as the acid is gradually volatilized. One hour's heating at 90–95° C. is necessary to drive off all of the acetic acid, after which normal figures for all of the determinations may be obtained.

CONCLUSIONS

I—Fats separated from dairy products by decomposition with sulfuric or hydrochloric acid will give the correct results for all of the usual determinations without any further treatment.

II—Fats separated in the presence of acetic acid will retain this acid and cause erroneous results. The acid is retained mechanically and must be entirely removed by drying for one hour at 90–95° C. before proceeding to determine the constants.

Chemical Laboratories, Department of Health
Cincinnati, Ohio

STUDIES IN SYNTHETIC DRUG ANALYSIS
IV—ESTIMATION OF PHENACETIN AND SALOL IN ADMIXTURE

By W. O. Emery, G. C. Spencer and C. C. LeFebvre

Received April 7, 1915

INTRODUCTION

In the course of certain analytical work on medicinal tablets, we were considerably handicapped for a time through lack of a quantitative method for estimating phenacetin and salol in admixture. Accordingly, efforts were directed first to their separation and second to their individual determination. It was found that a satisfactory separation could be effected by treatment either with dilute sulfuric acid or with sodium hydroxide of definite concentration, followed by extraction with chloroform.

The separation proper, therefore, follows two distinct lines of procedure, involving on the one hand the acid hydrolysis of phenacetin, on the other the alkaline hydrolysis of salol. In the first instance, the direct estimation of phenacetin only is contemplated, the salol being determined by difference in all cases where the combined weight of both constituents is known or can be conveniently ascertained. In this procedure, the phenacetin is temporarily converted into phenetidine sulfate and acetic acid, while in the case of salol partial volatilization results during the process of digestion. The phenetidine salt, by virtue of its insolubility in chloroform, is readily freed from further contaminating salol by treatment with that solvent, then by means of acetic anhydride and sodium bicarbonate reconverted into phenacetin, the latter being subsequently isolated and weighed.

In the case of alkaline hydrolysis, the salol is first converted into sodium salicylate and phenolate,